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TITLE: Defining Leukemia Stem Cells in Acute Lymphoblastic Leukemia

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CONTRACTING ORGANIZATION: Boston Children's Hospital

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14. ABSTRACT

Since their discovery, leukemia stem cells (LSCs) have been an intensive focus of investigation, particularly in acute myeloid leukemia (AML), with the development of several new LSC-targeting therapies currently in clinical trials for AML. Preliminary studies from our group and others indicate that ALL LSCs are heterogeneous with respect to their differentiation states, suggesting that they may be difficult to target therapeutically. Moreover, in our preliminary studies, we find that ALL LSCs are plastic – they are present in multiple lymphoid progenitor-like states, and LSCs from each state can reconstitute the entire cellular diversity of ALL. Using primary human patient ALL cells and patient-derived xenografts (PDXs), we find that as the most differentiated LSCs upwardly reconstitute ALL upon xenotransplantation, they engage oxidative metabolic pathways. This objective of this proposal is to perform the preclinical studies necessary to determine whether targeting mechanisms regulating ALL LSC plasticity represents a viable approach to therapy. In this proposal, we aim to test our hypothesis that ALL LSCs alter their metabolic activity to regulate cellular plasticity.

15. SUBJECT TERMS

NONE LISTED

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Introduction

The project is focused on the biology of acute lymphoblastic leukemia (ALL), particularly leukemia stem cells (LSCs). Prior to our work, it was generally accepted that ALL LSCs are heterogeneous, existing in multiple states of differentiation, and therefore, they were difficult pinpoint to assign their properties and identify potential vulnerabilities. In our preliminary studies, we used single cell RNA sequencing (scRNA-seq) to define the steady-state ALL cellular hierarchy and identify a fraction of *MLL*-rearranged B cell ALL (B-ALL) enriched in LSCs. We functionally validated this stem cell enriched fraction using quantitative xenotransplantation. Through transcriptomic and follow-up functional studies, we identified heterogeneity in the metabolic states of B-ALL blasts, with LSCs assuming a hypoxic and glycolytic state, akin to normal long-term hematopoietic stem cells (HSCs), while expanding, non-LSC blasts and leukemic progenitors showed signatures of oxidative metabolism. We found that targeting hypoxic signaling with the chemotherapy drug echinomycin depleted LSCs in vivo. Since these studies were focused on *MLL*-rearranged B-ALL – a high-risk form of ALL biased toward young children – our current studies focus on advancing this research in two ways: 1) by understanding the properties of LSCs in other high-risk forms of ALL with wider age distributions to query hypoxia and glycolysis as a vulnerability, and 2) determine mechanisms of LSC plasticity mediated by shifts in metabolic state.

Keywords
Leukemia
Stem cells
Metabolism

Accomplishments

What were the major goals of this project?

1. Determine the role of oxidative metabolism in LSC plasticity in ALL;
2. Examine the effects of targeting oxidative metabolism in human models of ALL.

What was accomplished under these goals?

In our preliminary data for this proposal, we found that phenotypically differentiated, CD34- B-ALL blasts could give rise to LSCs that had the capability of re-establishing the entire B-ALL cellular hierarchy. We observed that these reconstituting CD34- blasts (34-r cells) activated signatures of oxidative phosphorylation and showed evidence of high mitochondrial function relative to other leukemic fractions (see Figures 7-8 of original proposal). These findings form the basis for examining oxidative metabolism as a potential mediator of LSC plasticity and possible vulnerability in B-ALL. As documented in last year's report, we published a high-impact paper on this work, and so our current studies now focus on initiating the next phase of this work in other subtypes of B-ALL as well as mechanistic studies aimed at determining how metabolic and stem cell programs interface in B-ALL.

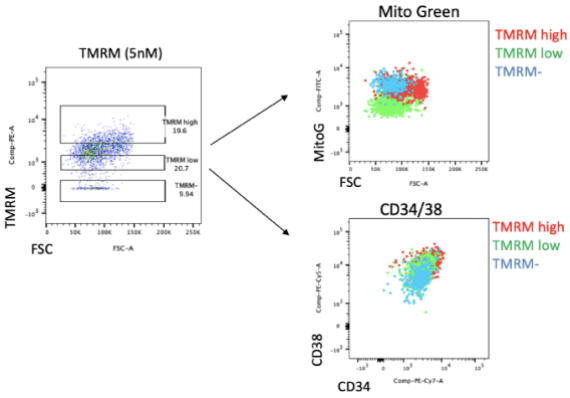

Figure 1. A *KMT2A*-rearranged B-ALL specimen was stained for TMRM (mitochondrial polarization), Mitotracker green (mitochondrial mass) and the surface markers CD34 and CD38. This result demonstrates that B-ALL cells are heterogeneous in terms of their mitochondrial metabolism, with low-polarized cells tending toward lower CD38 expression which is consistent with an LSC immunophenotype.

In our work published last year and show in last year's progress report, we showed that the aggressive subset of *KMT2A*-rearranged pediatric B-ALL was sensitive to pharmacologic inhibition of transcription by hypoxia inducible factor-1α (HIF-1α) in vivo. Since this is a small subset of ALL overall, out current efforts toward goal #1 now aim to generalize these results to other forms of aggressive ALL in adults and children. Through various collaborations, we have assembled a cohort of high-risk B-ALL subtypes including *BCR-ABL*/Philadelphia chromosome (Ph^+) B-ALL from adults and additional *KMT2A*-rearranged B-ALL and trisomy 21-associated B-ALL from children. We find that these aggressive subtypes of leukemia show heterogeneity in their metabolic profiles, consistent with potentia l LSC subpopulations with low levels of mitochondrial metabolism (Figure 1). Consistent with our hypothesis that LSCs assume a metabolic state of low mitochondrial metabolism, we used fluorescence activated cell sorting to isolate B-ALL cells of low mitochondrial mass versus high using the Mitotracker dye (Figure 2). We xenotransplanted these cells into immunocompromised NSG mice, finding that Mitotracker-low cells more efficiently engrafted mice with lower latency of terminal leukemia, overall consistent with a higher LSC content (Figure 2). However, Mitotracker-high cells eventually cause terminal leukemia, consistent with the plasticity in B-ALL cell identity that we and others have previously observed (Figure 2). We currently have CUT&RUN data pending which aims to understand at what sites in the genome HIF-1α is binding to regulate stem cell programs in B-ALL cells.

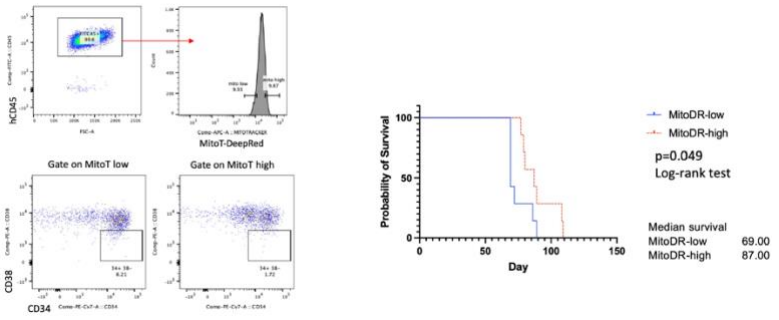

Figure 2. A *KMT2A*-rearranged B-ALL specimen was stained for Mitotracker green (mitochondrial mass) and low- versus high-staining cells were xenotransplanted into immunodeficient NSG mice with survival monitored. Survival curves were compared by log-rank test.

In terms of goal #2, so far, we have tested the effect of the HIF-1α inhibitor echinomycin on B-ALL growth in vivo in two independent Ph^+ specimens, finding that this intervention diminishes leukemic growth in vivo (Figure 3). We are testing additional Ph^+ specimens from adults, as well as trisomy 21-associated B-ALL and *KMT2A*-rearranged B-ALL from children.

With respect to the Statement of Work, our current progress for Aim 1 is: Major Task 1, Subtasks 1-5. We are currently completing Subtask 6 – these were slightly delayed as we were completing the revisions for our publication. We are in the process of performing the studies for Major Task 2 as part of our mechanistic work. We have nearly completed Major Task 3, Subtask 1. For Aim 2, we have nearly completed Major Task 2, Subtask 1.

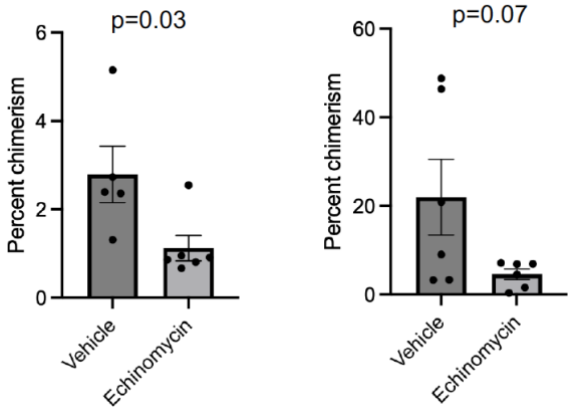

Figure 3. Ph^+ B-ALL cells were engrafted into NSG mice. Three weeks later, treatment with the HIF-1α inhibitor echinomycin (10 μg/kg/dose) for 10 days was initiated. Mice were then euthanized and human chimerism in the bone marrow was quantified by flow cytometry and results compared by student's t-test.

What opportunities for training and professional development has the project provided?

1. Please describe your Year 1 CSVCC activities.

   We have integrated with the CVSCC leadership and scholars. Dr. Rowe has met twice for one-on-ones with CVSCC leadership and other scholars. He has achieved a DAPER grant with Dr. Abby Green for a new collaboration which is ongoing. He is also actively collaborating with Dr. Peiwei Chen and is a co-author on one of Dr. Chen's submitted manuscripts. He is also collaborating with Dr. Gryder to look at chromatin changes in normal hematopoietic maturation.

3. Discuss how your participation in the CSVCC is allowing you to engage with patient communities or organizations.

   Participation with the CVSCC has led to introducing us to military health advocates and consideration of the impact of our research on these communities. Participation in the CVSCC conferences has been key to these learning experiences.

4. Discuss how your participation in the CSVCC has impacted your career goals as outlined in the Career development Plan and your research project.

   Participation with the CVSCC has supported the development of my laboratory, particularly allowing us to complete a publication based on this work last year and to advance the current studies. This funding has been vital for us to establish ourselves and support our early research.

5. Discuss how your participation in the CSVCC is allowing you to learn about the impact of cancer on Military Health and mission readiness.

   Participation with the CVSCC has led to engagement with military health advocates and tailoring of our research to support Military Health and mission readiness. We have broadened our horizons beyond our initial focus on childhood leukemia to become interested in the biology of leukemia across the age spectrum.

How were the results disseminated to communities of interest?

Nothing to Report – work is in progress.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to analyze our CUT&RUN date to determine the mechanisms by which hypoxic metabolism regulate the stem cell state. We plan to perform mechanistic studies to determine how mitochondrial turnover regulates B-ALL cell state plasticity and the relevance of this process to the LSC state.

Impact

What was the impact on the development of the principal discipline(s) of the project?

This research has identified a new therapeutic approach to treatment of *MLL*-rearranged B-ALL, and aggressive subtype of acute leukemia that is skewed toward infants and young children but can affect people of all ages. This has the potential for clinical translation to benefit patients. We have preliminary data to indicate that this intervention might also benefit older patients with high-risk B-ALL subtypes.

What was the impact on other disciplines?

Our approach to identifying mechanisms of phenotypic plasticity in leukemia is potentially applicable to the study of other types of tumors. We have published our initial findings last year and it remains to be determined how these results will impact the broader cancer stem cell field.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

Changes/problems

Change in approach and reasons for change

Since our original proposal, our studies have identified a role for hypoxic/glycolytic metabolism in ALL LSC plasticity, and so we are incorporating study of this pathway into our Aims and Statement of Work. This does not change our overall plan and schedule.

The panel of high-risk B-ALL specimens was somewhat constrained to what is available – we believe that the specimens ascertained are representative of high-risk subtypes.

We have also gained recent expertise in CUT&RUN – this is a new opportunity to understand how HIF-1α and we are planning on this mechanistic experiment.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Products

Publications, conference papers, and presentations

Journal publications

Morris V, Wang D, Li Z, Marion W, Hughes T, Sousa P, Harada T, Ho Sui S, Naumenko S, Kalfon J, Sensharma P, Falchetti M, Vinicius da Silva R, Candelli T, Schneider P, Margaritis T, Holstege FCP, Pikman Y, Harris M, Stam RW, Orkin SH, Koehler AN, Shalek AK, North TE, Pimkin M, Daley GQ, Lummertz da Rocha E, **Rowe RG**. Hypoxic, glycolytic metabolism is a vulnerability of B-acute lymphoblastic leukemia initiating cells. *Cell Rep* 39, 110752 2022.

Presentations

The research from this project was presented at the International Society for Hematology annual meeting in September 2021.

Websites(s) or other internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other products

Nothing to Report.

Participants and other collaborating organizations

What individuals have worked on the project?

Name:	R Grant Rowe
Project Role	Principal investigator
Researcher identifier	0000-0003-3620-2950
Nearest person month worked	5.4
Contribution to project:	Dr. Rowe has supervised, designed, and executed the research.

Name:	Dahai Wang
Project Role	Research technologist
Researcher identifier	
Nearest person month worked	12
Contribution to project:	Dr. Wang has executed the research.

Name:	Chun-Chin Chen
Project Role	Postdoctoral fellow
Researcher identifier	
Nearest person month worked	12
Contribution to project:	Dr. Chen continues to execute this project.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Fanconi Anemia Research Fund – closed.

What other organizations were involved as partners?

Nothing to Report.